**Schedule for carba NAD solubility studies:**

**1) Dalton:**

* Previous proposal:

“Dalton proposes to prepare a stock solution of Carba-NAD in water at a concentration of 3mM or higher, if possible. Dalton will use 10mg Carba-NAD (L) KF-01-163-P01-012417 for preparing the stock solution. Small aliquots of stock solution will be diluted with buffer solution (with higher concentration) to achieve desired buffer concentration and visually inspect for Carba-NAD precipitation. Dilution experiments will be carried out for a final Carba-NAD concentration of 1mM, 0.3mM and 0.1mM concentration in buffer. Results from these experiments will be summarized in a brief report.”

* Cost: $7625
* New proposal:

“I will prepare a proposal for the scope described below and forward it to our Chemistry team for review”

Excluding the cost for 5 mg carba NAD ($1400), what will be the price for the studies based on the points below?

a) We will send you the buffer (50 mM Tris-HCl, 137 mM NaCl, 2.7 mM KCl, 1 mM MgCl2, 5% DMSO, pH 8.0), so you don’t have to charge us for costs associated with this.

b) We would like you to do the minimum number of dilutions and HPLC runs required to determine the maximum concentration.

* Cost: Not yet available. Will be available early next week.

**2) Ascendex:**

* The following email was sent to Ascendex regarding the solubility studies:

“Dr Gao Shang

Would you please provide a quote for the Solubility of Carba-NAD in 5%DMSO-HDAC solution ?

We are going to provide the Carba-NAD and HDAC buffer. Please include the following into your quote

(1) The minimum amount of Carba-NAD needed for the solubility test.

(2) The timeframe”

* Cost: For the previous DHP/HKL solubility study they charged $2000 for the solubility studies only.

Previous quote: “Solubility measurement of two compounds, in buffer, 1%, 2% and 5% DMSO in water at ambient temperature. HPLC measurement by calibration curve. All materials (25 mg of each compound, 40 mL buffer) are supplied by the client.”

* Estimated timeframe: 1-2 weeks

**3) In-house:**

* If we need to do the solubility studies in-house, we could follow the procedure sent to us by Ascendex previously.
* Summary of procedure:

a) Calibration curve:

- Stock solutions will be prepared by weighing an exact amount of sample in 5% DMSO-HDAC buffer.

- Various dilutions of the stock solutions will be made accurately.

- The dilutions will be analyzed by HPLC

- A linear calibration curve will be established

b) Sample preparation:

* A series of over saturated solutions will be prepared and equilibrated for 24 hours and visually observed.
* The solutions will be filtered by a membrane filter and analyzed by HPLC
* Concentration will be determined by referencing to the calibration curve.
* We will first wait for quotes from Dalton and Ascendex and if both are unacceptable, then we will proceed to do these studies in-house